Abstract
Cancer chemotherapy can produce severe side effects such as suppression of immune function and damage to heart muscle, gastrointestinal tract, and liver. If serious enough, tissue injury can be a major reason for late stage termination of drug discovery research projects, so it is becoming more important to integrate safety/toxicology assessments earlier in the drug development process. There are a variety of traditional serum markers, tailored mechanistically to specific tissues, however there are no current non-invasive assessment tools that are capable of looking broadly at in situ biological changes in target and non-target tissue induced by chemical insult.

We used non-invasive biodistribution imaging (IVIS SpectrumCT, PerkinElmer Inc.) and near infrared (NIR) fluorescence tomography (FMT® 4000, PerkinElmer Inc.) for whole-body imaging of luciferase-expressing HT-29 human colon adenocarcinoma tumors implanted in nude mice. Both tumor and host tissue responses were imaged using a cocktail of near infrared fluorescent imaging agents, specific for cell death (Annexin-Vivo 750™ [AV750]), inflammatory matrix metalloproteases (MMMPsense™ 750 FAST™ [MMP750]), and metabolic changes in transferrin receptor expression (Transferrin-Vivo™ 750 [TIV750]). Vascular changes were imaged using AngioSense® 680 (A680). As a means of validating this efficacy/biodistribution screening approach, HT-29-bearing nude mice were dosed with 5-Fluorouracil (5-FU). 5-FU has been a mainstay in the treatment of many cancers, including colorectal, but is associated with several peripheral toxicities, including gastrointestinal, hepatic, renal, vascular, and (less frequently) cardiac. Dosing was performed as a single IP bolus administration, using doses (50 and 100 mg/kg) known to have minimal overt effects on body weight or tumor mass using this acute dosing regimen. At 2h and 24h post-5-FU, independent cohorts of mice were injected with the AV750/MMP750/TIV750 750 cocktail (AMT 750) combined with A680. Imaging was performed 24h later. No apparent effects were seen on tumor mass with this very short treatment regimen, although there was a trend for decreased biodistribution. 2h following 5-FU, however both tumor vascular leak and AMT750 signal increased at the high 5-FU dose. Tumors, heart, liver and lungs showed predominant changes in AMT750 signal, with the heart showing the most dramatic increase (>20-fold). The acute nature of the response, and the absence of histologic inflammation, suggests that AMT750 was detecting tissue apoptotic changes, perhaps in the vascular endothelium.

The stomach, kidneys, and Intestines showed predominant increases in A680, indicating changes in vascular permeability in these tissues (known to occur with 5-FU). The reversible nature of the biological changes at 24h suggests that this could be a sensitive imaging approach for detecting early tissue toxicity. These results agree well with observations in the literature that have seen 5-FU effects in the same tissues in both preclinical studies and in humans undergoing treatment.

1 Biodistribution and Tox/Efficacy Imaging Protocols

A. Mouse Tumor Model

B. Whole Body Fluorescence Tomography

2 Validating a Cocktail of Imaging Agents for Detection of Patterns of Drug-Induced Tissue Injury

A. Epifluorescence Screening for Tissue Injury using the imaging cocktail AMT750

Non-Invasive IVIS Imaging & Quantification

Ex Vivo Tissue Quantification

RMP

TAA

Normal BALB/c female mice were injected IP with a single 100 mg/kg dose of mitomycin C (MMC) or doxorubicin (Dox), known to induce toxicity in various tissues. The AMT 750 cocktail was administered 24h after drug dosing and mice were imaged on the IVIS SpectrumCT by epifluorescence. In vivo images revealed increased signal in the liver region, and an ex vivo tissue imaging identified the specific tissue localization of signal indicative of bioluminescent change (hepatic, heart, kidneys, etc.). Note the different tissue profiles induced by 2 different toxic drugs.

3 Noninvasive Imaging of Anti-Tumor Efficacy and Biological Effects on Normal Tissue Simultaneously

A. 5-FU Treatment: Tumor BLI

B. 5-FU Treatment: FL Tomography

4 Quantification of Single Dose 5-FU Tumor Efficacy and Profiling for Potential Toxicity in Normal Tissues

A. IVIS Spectrum BLI

B. B. FMT 4000 Quantification

C. Reversibility of 2h 100 mg/kg 5-FU Effects on Tissues 24h After Treatment

5 Biological Changes in the Absence of Histologic Changes

After the animals were imaged in vivo, they were sacrificed by carbon dioxide asphyxiation. The organs (brain, heart, lungs, liver, pancreas, spleen, stomach, intestines, kidneys, fat, skin, and tumor) were removed post-mortem and fixed with 10% formalin for histological assessment by H&E. No overt evidence of toxicity was seen despite measurable BLI decreases in tumors from treated animals and biomarker changes in tumors, heart, liver, lungs, stomach, kidneys, and intestines.

Summary
The present studies provide evidence for the utility of a cocktail of imaging agents, detecting cell death, MMP activity, and transferrin receptor upregulation, in the detection of both acute drug-induced tissue changes as well as anti-tumor efficacy. A variety of toxicities are associated with 5-FU treatment regimens, including oral and gastrointestinal mucositis, stomach pain, nephrotoxicity, hepatotoxicity, and cardiotoxicity. It is remarkable that in these studies as little as a single administration of 5-FU induced detectable biomarker changes in tissues previously characterized to show injury following much longer-term 5-FU dosing regimens. Many of these toxicities are poorly understood in patients and very difficult to recapitulate in mouse tumor models, however optical imaging could readily detect biological changes in the absence of overt histologic changes.

In conclusion, imaging fluorescent biomarkers of tissue biological changes should provide a robust approach to the simultaneous assessment of drug-induced efficacy and tissue injury in mice, allowing early screens of therapeutics in drug discovery.